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# USCAP & AACR HIGHLIGHTS

## Nuevas tecnologías

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USCAP & AACR HIGHLIGHTS 2012

## [2152] Next-Generation Pathology: Deep DNA Sequencing and Targeted Therapy for Cancer

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- **Background:** Gene sequencing currently used to select therapy in non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and melanoma (MM) employs traditional standard-of-care (SOC) "hotspot" single gene mutation analysis. Massively parallel (next-generation sequencing (NGS)) has now been adapted to formalin-fixed (FFPE) specimens to provide high sensitivity detection for point mutations, insertion/deletions, translocations and copy number alterations (CNA).
- **Design:** FFPE specimens from 83 solid tumors (50 CRC, 29 NSCLC, 4 MM) with results available from SOC genotyping by allele-specific PCR (KRAS codons 12/13, EGFR exons 17-20, or BRAF V600E) were fully sequenced for 145 genes by NGS. Hybridization-capture of 2574 exons across 145 oncogenes, tumor suppressor genes and ADME-related genes was performed to produce libraries appropriate for paired-end sequence analysis on the Illumina HiSeq2000 platform (Illumina, Inc., San Diego, CA).
- **Results:** NGS recapitulated the SOC test results in all cases. In-depth sequence analysis with median coverage averaging 213-fold (range 8 to 461) detected a per-sample average of 2 previously-described mutations, 7 novel mutations and 2 CNAs in the CRC, including frequent alterations in *TP53* (33), *APC* (27), *KRAS* (12) and *BRAF* (6). The NSCLC averaged 1 previously described mutation, 8 novel mutations and 1 CNA per sample, most frequently *KRAS* (10), *TP53* (7), *JAK2* (3), *EGFR* (2) and *BRAF* (2). The MM exhibited on average 1 previously described mutation, 7 novel mutations and 3 CNAs including *TP53* (4) and *BRAF* (2). In addition to validated clinically actionable mutations in *EGFR*, *KRAS,* and *BRAF*, and multiple alterations in well-known cancer genes (*TP53, STK11, APC, MLH1, BRCA2, SMAD4*), a significant number of additional genomic alterations that have potential therapeutic implications were also detected including activating mutations in the PI3 kinase subunit gene *PIK3CA*; mutations in *MET, KIT, ERBB2* and *CDKN2A*; driver mutations not usually associated with solid tumors, such as the lymphoma-associated *JAK2* V617F mutation in two NSCLCs; and in 1 CRC, a novel chromosome 2 rearrangement adjacent to the *ALK* kinase domain confirmed by analyzing a cDNA library constructed from extracted tumor RNA.
- **Conclusions:** NGS of hundreds of cancer-related genes can be reliably performed at a high level of sensitivity and specificity in clinical FFPE samples of solid tumors, can reproduce SOC single gene traditional sequencing results and shows great potential to inform on therapeutic decisions for patients with CRC, NSCLC and MM.

Category: Techniques

## [2140] Mutational Screening in *KRAS, BRAF, EGFR, C-KIT* and *PDGFRa*in Colorectal Carcinoma (CRC) and Non-Small Cell Lung Cancer (NSCLC) Using Next-Generation Sequencing (NGS)

***Tanja Hinrichsen, Oliver Wachter, Barbara Dockhorn-Dworniczak, Hanns-Georg Klein. Center for Human Genetics and Laboratory Medicine Dr. Klein and Dr. Rost, Munich, Bavaria, Germany; Institute of Pathology Kempten, Kempten, Bavaria, Germany***

- **Background:** Treatment with monoclonal antibodies (mAB) or „small molecules" (e.g. tyrosine kinase inhibitor, TKI) depends on the mutational status of certain genes in the tumour tissue of solid tumours. In CRC, the mutational status of *KRAS* and *BRAF* correlates with a response to mABs, in NSCLC the mutational status of EGFR and KRAS correlates with a benefit to TKI treatment. Other important genes in gastrointestinal stromal tumour (GIST) are *c-KIT* and *PDGFRa*. With NGS, is likely to improve the diagnostics in solid tumours. Discrete templates of the DNA can be amplified and sequenced clonally with a high coverage in a time-saving and cost-efficient manner. Mutations can be detected in a wildtype-background which enables the detection of minorities and is another important step towards personalized medicine.
- **Design:** NGS was applied for a molecular screening of the complete coding region of *KRAS, BRAF, EGFR, C-KIT* and *PDGFRa* to investigate for mutations in FFPE-tumour tissue. A set of 48 CRC-FFPE-samples and a set of 48 NSCLC-FFPE-samples were analyzed. DNA was isolated and amplicon preparation (300 bp) was automated with the Fluidigm Access Array System (Fluidigm, South San Francisco, CA). Four Access Arrays were performed and multiplex Identifiers (MIDs) were added manually. After pooling and purification the pooled library was sequenced with GS-FLX (454 Life Sciences, Branford, CT, USA) with an aimed 200xcoverage.
- **Results:** For the NSCLC-set, 544777 reads which passed filter criteria were generated with an average coverage of approximately 118x. First results show mutations in *KRAS* in the known hotspot regions in a subset of *EGFR*-negative NSCLC-patients. For the CRC-set, 720509 reads which passed filter criteria were generated with an average coverage of approximately 156x. The analysis of the data is still going on. Data for both runs will be presented.
- **Conclusions:** The combination of the GS FLX and the Access Array System could offer a high-throughput amplicon resequencing solution applicable on DNA from FFPE-tissue. Our data suggest that NGS is a feasible method in routine molecular pathology to examine mutational status of tumour-relevant genes which are the basis for personalized medicine.

Category: Techniques

## [2158] Ultra-Rapid Diagnostic Tissue Preparation as an Alternative to Frozen Section

***Victoria Sujoy, Clifford Blieden, Monica Garcia, Steven E Vernon, Azorides R Morales. University of Miami Miller School of Medicine, Miami, FL; Jackson Memorial Hospital, Miami, FL***

- **Background:** Hardening the tissue by freezing, which eliminates the need for fixation, processing and embedding, was introduced as a method for intra-operative pathologic consultation by Louis B Wilson at the Mayo Clinic in 1905. Because of artifacts induced by freezing tissue these intra-operative diagnoses are not uncommonly discordant with that provided by preparation of paraffin embedded blocks from the same frozen tissue (necessary to confirm the pathologic diagnosis rendered during surgery, and also for laboratory accreditation). We sought to determine if modifying conventional histological procedures could allow preparation of H&E sections from paraffin blocks in less than 20 minutes from receipt of tissue in the laboratory.
- **Design:** We developed new tools and grossing procedures to facilitate very thin slicing of fresh tissue, designed a chemical admixture of a ketone, mineral oil and surfactant to use during grossing and microwave-based processing for 8-10 minutes (Sakura's Xpress 50), and modified procedures to shorten paraffin embedding, microtomy, staining and coverslipping to no more than 7 minutes instead of more than 30minutes as is customary. Fresh samples from a variety of different residual tissues and diseases were subjected to this novel tissue preparation method. In addition to H&E, panels of most common histochemical and immunohistochemical stains were done.
- **Results:** The histomorphology of H&E stains obtained with this system is indistinguishable from conventional diagnostic tissue preparation. Moreover, histochemistry and immunohistochemistry results are similar, if not identical, to those obtained with formalin-fixed conventionally processed tissues.
- **Conclusions:** Tissue sections from blocks prepared with this system have morphologic characteristics of similar or identical quality when compared with paraffin blocks prepared by conventional processing. This is a significant improvement over examination of a conventional frozen section, does not suffer from the morphologic artifacts of the latter, precludes the need to process previously frozen tissue and provides intra-operative "permanent section" diagnosis. Moreover, preliminary studies suggest that the preservation of RNA in paraffin blocks obtained with this system is similar to that from fresh frozen tissue.

Category: Techniques

## [1901] A Novel microRNA-Based Test Demonstrate above 90% Accuracy in Classification of Metastatic Tumors from Patients Diagnosed with Carcinoma of Unknown Primary

***Mats Sanden, George Pentheroudakis, Brianna St. Cyr, Anna Goussia, Danit Lebanony, Katerina Stoyianni, Alexander Faerman, George Fountzilas, Lahav Cohen, Vassiliki Malamou-Mitsi, Nicholas Pavlidis. Rosetta Genomics Inc., Philadelphia, PA; Ioannina University Hospital, Ioannina, Greece; Rosetta Genomics Ltd., Rehovot, Israel; Hellenic Cooperative Oncology Group, Athens, Greece***

- **Background:** Identification of the tissue of origin of metastatic tumor is vital to its management. Carcinoma of unknown primary (CUP) is common in oncology, representing 3-5% of all invasive malignancies. A microarray-based test that measures the expression of 64 microRNAs was employed to identify the tissue of origin of metastatic tumors of CUP cases.
- **Design:** A cohort of resected metastatic lesions from patients diagnosed with CUP was studied. The cohort included 93 samples (from 92 patients) with adequate tissue sample needed for the test. Eight samples failed due to inadequate RNA quality; 85 samples (84 patients) were processed successfully. Test results were compared with clinical presentation including imaging, pathological data (histology and IHC) and therapeutic response.
- **Results:** In this blinded study, the test results were fully concordant with the diagnosis based on all the clinical and pathological information available including follow-up and outcome data in over 90% of the cases. The microRNA test assigned a single putative tissue of origin for 50 samples and two tissues of origin in 34 patients with the first being the more likely diagnosis. When comparing only the first (or single) diagnosis, a concordant level of >83% is achieved. The diagnosis based on the clinical and pathological data that was available at presentation and without additional data gathered throughout patient management had only 70% agreement with the test results. Additional clinical and pathological analysis of the CUP cases is currently ongoing.
- **Conclusions:** In a cohort of metastases from CUP patients, a previously developed test based on the expression profile of 64 microRNAs allowed accurate identification of tissue of origin in the vast majority of the cases. The high accuracy of this test in identifying the tissue of origin of metastasis of unknown primary has been validated by this study and demonstrates its clinical utility. The high concordance of the test results to the final diagnosis of the patient demonstrates the importance of the test to yield additional data valuable for patient's management at an early stage of patient's journey.

Category: Special Category - Pan-genomic/Pan-proteomic approaches to Cancer

## [1899] Identification of Microvascular Invasion Biomarkers in Hepatocellular Carcinomas by MALDI Imaging Mass Spectrometry

***Nicolas Pote, Theodore Alexandrov, Samira Laouirem, Jacques Belghiti, Pierre Bedossa, Valerie Paradis. Beaujon Hospital, Assistance Publique-Hôpitaux de Paris, Clichy, France; INSERM U773, Beaujon Hospital, Clichy, France; University of Bremen, Bremen, Germany; University Denis Diderot, Paris, France***

- **Background:** Microvascular invasion (mVI), a major predictive factor of post-operative tumoral recurrence in patients with hepatocellular carcinoma (HCC), is only detectable after surgery on histological examination of the surgical specimen. So far, there is no reliable biomarker to predict the presence of mVI prior to surgical procedures. MALDI Imaging Mass Spectrometry (IMS) represents a new analytical tool to directly provide the relative abundance and spatial distribution of the whole proteins expressed in a tissue section. The aim of this study was to compare, using MALDI IMS, the tissue proteome of HCC without and with mVI in order to identify biomarkers of mVI.
- **Design:** A total of 56 HCC from various etiologies obtained from surgical specimen, for which clinicopathological data and frozen samples were available, were retrospectively collected. All patients were cirrhotics, and none of them received any neo-adjuvant treatment. After histological examination, two groups of tumors were defined (26 HCC without mVI; 30 HCC with mVI). Cryosectioning was done to yield tissue sections analysed by a pathologist to determine tissue morphology and mirror sections for MALDI IMS. A statistical comparative analysis, using a cross classification model, was then performed in order to identify protein peaks differentially expressed between both groups.
- **Results:** 24 discriminant protein peaks were differentially expressed between both groups, of which 11 were discriminant in more than 30/56 cross classifications. 2 peaks increased in HCC without mVI, whereas 22 increased in HCC with mVI. The latter, which could be used as tissue mVI biomarkers, are under selection for protein characterization. Tissue distribution analysis of the two most discriminant peaks (*m/z* 10,042 and 8,904) showed that *m/z* 10,042 was preferentially expressed in tumor cells, whereas *m/z* 8,904 was overexpressed in the stroma. Interestingly, we have previously described *m/z* 8,904 as a serum biomarker of HCC (Paradis et al., Hepatology 2005).
- **Conclusions:** These results highlight the potential of MALDI IMS to uncover new biomarkers in liver carcinogenesis, and to allow their tissue localization. The identification of mVI biomarkers would be helpful in the therapeutic strategy of patients with HCC.

Category: Special Category - Pan-genomic/Pan-proteomic approaches to Cancer

## [1931] Cytoplasmic Staining Pattern of Cyclin E and pCDK2 Expression Correlates with Poor Outcome in Breast Cancer (BC) Patients (pts)

***Cansu Karakas, Anna Biernacka, Aysegul Sahin, Kelly Hunt, Khandan Keyomarsi. MD Anderson Cancer Center, Houston, TX***

- **Background:** Cyclin E, a key regulator of the cell cycle, and its co-activator pCDK2 play an important role in BC biology. Recently, we showed that high levels of cyclin E expression are associated with poor prognosis in BC. Guidelines for cyclin E evalution is lacking. The aim of this study was to identify a biologically significant evaluation method for cyclin E and pCDK2.
- **Design:** Immunohistochemical (IHC) analysis of cyclin E (Santa Cruz 1:1000) and p-CDK2 (Cell Signaling 1:100) was performed on archival sections from 209 pts with stages I-III BC. Cyclin E staining intensity and percentage of positivity were evaluated in the nucleus (N) and cytoplasm (C) and four patterns were distinguished: negative, predominantly N, both N and C and predominantly C. pCDK2 staining was also evaluated in the N and C. Results was correlated with disease-specific survival (DSS).
- **Results:** Among the 209 pts, 119 (57%) showed C cyclin E positivity which was associated with poor outcome compared with outcome in those with C cyclin E-negative tumors. Recurrence was observed in 26/119 pts whose tumor showed C staining but was observed in only 5/90 pts whose tumors showed N staining which was statistically significant (p=0.01). Among the 119 pts with C cyclin E positivity 66 had tumors that were both N and C cyclin E-positive, and 53 had tumors that were only C cyclin E-positive. Recurrence was observed in 14/66 pts whose tumors were both N and C cyclin E-positive, and in 12/53 pts whose tumors were only C cyclin E-positive. Although, both of these groups were associated with poor DSS, the C cyclin E-positive group was more significantly correlated with poor DSS (p=0.006). Furthermore C pCDK2 expression was also associated with poor prognosis. Recurrence was observed in 18/75 pts whose tumor had C pCDK2 staining versus 13/134 pts whose tumor shows no C staining (p=0.004). Pts with tumors positive for both C cyclin E and pCDK2 had the highest recurrence rate (p=0.016).
- **Conclusions:** IHC is a clinically valid method for assessing Cyclin E. C pattern identifies pts with poor prognosis who may benefit from investigational treatment strategies such as CDK2 inhibitors.

Category: Pathobiology

## [1944] Molecular Detection of Metastatic Cancer in Cell-Free Cytocentrifugation Supernatant Fluid from Needle Aspirates of Lymph Nodes

***Allan R Smith, Jan F Silverman, Yulin Liu, Uma Krishnamurti, Shahid Bokhari, Candy Binkert, Beth Ujevich, Alok Mahonty, Sidney D Finklestein. Allegheny General Hospital, Pittsburgh, PA; RedPath Integrated Pathology, Inc., Pittsburgh, PA***

- **Background:** Cytologic diagnosis of lymph node needle aspirates for the presence or exclusion of metastatic cancer can be difficult leading to occasional indeterminate diagnosis especially if only a few atypical cells are present. We explored whether cell free DNA from the lymph node aspirate could serve as an independent source for mutational analysis with the goal to improve the detection or exclusion of malignancy.
- **Design:** 8 needle aspirate of lymph nodes with metastatic cancer (n=4) and without cancer (n=4) were used in this pilot study. In each case, DNA was extracted from 2 ml of cell-free cytocentrifugation supernatant fluid from residual aspirate not utilized in preparing direct smears. DNA quantity measured by optical density. DNA amplifiability was assessed using qPCR. Mutational analysis followed using PCR/capillary electrophoresis for a broad panel of markers (KRAS point mutation by sequencing, microsatellite fragment analysis for loss of heterogeneity [LOH] of 16 markers at 1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q). In selected cases, microdissection of stained cytology smears and/or cytocentrifugation cellular slides were similarly analyzed and compared.
- **Results:** No detectable mutations were present in cytologic negative cases. All metastatic carcinoma cases showed extensive detectable mutations in both the microdissected tumor and corresponding cytocentrifugation supernatant fluid. The mutational profile between the malignant cells and supernatant was highly concordant including involvement of specific parental alleles when LOH was present. The supernatant fluid of all 4 cancer specimens showed additional mutations not present in the microdissected tumor. Also, for every mutation present in both specimen, the clonality of mutational change was equal to or higher in the supernatant fluid sample compared to the microdissected tumor cells.
- **Conclusions:** 1) The cytocentrifugation supernatant fluid, gathered during cytology preparation, and typically discarded, contains adequate levels of analyzable DNA suitable for mutation detection and characterization. 2) The greater content and higher clonality of mutational change in the supernatant fluid of lymph node aspirates affords a simple way to improve the detection or exclusion of cancer in cases with a limited number of atypical cells. 3) Molecular analysis of supernatnat fluid can be especially helpful for the detection of malignancy. The results support further studies to evaluate its clinical utility.

Category: Pathobiology

## [2126] Effects of Long Term Tissue Fixation on the Immunohistochemical Expression of MSI Makers in Colon Adenocarcinoma

***Patrick Adegboyega. LSU Health Sciences Center, Shreveport, LA***

- **Background:** Colorectal adenocarcinomas with microsatellite instability (MSI) do not respond well to Fluorouracil-based chemotherapy and do have treatment outcome that differs from that seen in microsatellite stable tumors. In clinical settings, immunohistochemical staining for makers of MSI [Mismatch repair (MMR) gene products] is used to screen for the presence of MSI; and has been shown to have comparable sensitivity and specificity with MSI detection by PCR. It was recently shown that routinely used tissue fixative such as dissect aid negatively impacts MMR proteins immunohistochemistry. 10% Neutral Buffered Formalin (NBF) was shown to be the optimal tissue fixative for MMR protein immunostaining in routine surgical pathology practice. This study explores the effects of long term NBF tissue fixation on the immunohistochemical expression of three MMR gene products (MLH1, MSH2 and MSH6).
- **Design:** Study materials consisted of cases of colonic adenocarcinoma: 7 primary colectomy and 1 secondary hepatectomy specimens received for tumor diagnosis and staging. Samples of normal colon and tumor from each specimen were fixed in NBF and submitted for routine processing with paraffin embedding after fixation for 1 day, 1 month, 3 months, 6 months and 1 year. Immunohistochemistry for MLH1, MSH2 and MSH6 was performed on representative sections of each block. Immunoreactivity scoring was done using a semi-quantitative score of 0, 1, 2, 3 and 4.
- **Results:** MLH1 immunoreactivity scores for all the samples were strong for the samples within the first 3 months of fixation (previous findings) but thereafter became drastically reduced and is completely negative in 5 of 8 cases for MLH1, 3 of 8 cases for MSH2 and 2 of 8 cases for MSH6 after one year of fixation.
- **Conclusions:** Although 10% Neutral Buffered Formalin solution is the preferred fixative for MMR immunohistochemical assay, long term tissue fixation (greater 3 months) results in loss of immunoreactivity of MMR proteins in tissue sections and so may produce spurious results (false MSI status).

Category: Techniques

## [2143] RNA from Archived FFPE Blocks – A Valuable Underexploited Resource

***Theresa J Kokkat, Virginia A LiVolsi, Miral Patel, Diane McGarvey, Munia Z Islam, Gina Piermatteo, Zubair W Baloch. University of Pennsylvania, Cooperative Human Tissue Network (Eastern Division), Philadelphia, PA***

- **Background:** Formalin Fixed and Paraffin Embedded (FFPE) tissue blocks constitute a valuable underexploited resource for molecular studies of disease. However the major limitation of FFPE samples for their use in molecular analyses is the degradation of RNA in FFPE samples. RNA Degradation can be caused 1) by various sample handling procedures prior to formalin fixation or 2) during formalin fixation which causes significant chemical modification of RNA or 3) after dehydration and paraffin-embedding where RNA continues to fragment and degrade over time.The quality of RNA can be represented either as RNA Integrity Number (RIN; Agilent Bioanalyzer) or RNA Quality Indicator (RQI; Biorad Experion) with a range of 1 to 10, representing totally degraded RNA to completely intact RNA. In FFPE samples, because RNA continues to degrade over time into small fragments, the RIN or RQI is much lower than frozen tissues. Recent improvements in the field of nucleic acid extraction as well as gene expression profiling have made it possible to study long-term archived tissues. Even a RIN of 1.4 has been successfully used for gene expression analysis. Therefore we investigated if time (in terms of years) of storage has effect on the quality of RNA on FFPE blocks. We compared RNA quality from FFPE blocks stored for 10 years, 5 years, 1 year to the blocks that have been stored for less than a year.
- **Design:** Lung FFPE blocks (10 years, 5 years, 1 year and less than a year, n=16) were obtained from either clinically archived or the Cooperative Human Tissue Network repository. Two to four 10 um curls were cut from each block and processed using Allprep DNA/RNA FFPE Kit from Qiagen. RNA quality was investigated using automated electrophoresis. A minimum RQI of 1.4 was considered as threshold for useful RNA quality.
- **Results:**

Table 1: RQI for blocks stored over different time periods

| Years Stored | # blocks analyzed | # cases >1.4 RQI | Mean ± S.D | Range |
|---|---|---|---|---|
| 10 years | 5 | 4 | 2.30 ± 1.10 | 1.0 -3.5 |
| 5 years | 4 | 3 | 2.43 ± 1.55 | 1.0- 4.6 |
| 1 year | 3 | 3 | 1.80 ± 0.00 | Not Applicable |
| less than 1 year | 4 | 4 | 2.58 ± 0.61 | 1.9 -2.8 |

Analyses of average RQI for FFPE blocks stored at different time periods did not show a significant difference between the blocks that were processed within several months before analyses and that had been stored for 10 years, 5years and 1 year

- **Conclusions:** RNA quality obtained from older series of FFPE blocks are comparable to more recent blocks indicating that RNA preservation and quality in FFPE blocks seems to be preserved over time up to ten years.

Category: Techniques

## [2146] Extraction and Molecular Screening of Decade-Old mRNA from Archived Breast Cancer Tissues

***David E Nowak, Leonardo P Roquero, Dhanajay A Chitale. Henry Ford Hospital, Detroit, MI***

- **Background:** Since the explosion of molecular techniques in pathology over recent years, a common goal has been to develop retrospective studies where patient outcomes are known. Abundant formalin-fixed paraffin embedded (FFPE) material is available at any institution generally archived for decades. Extraction of amplifiable mRNA from old blocks has been a challenge and has been sporadically reported. Our aim was to test mRNA quality extracted from archived FFPE blocks from breast cancer patients, where the inherent fatty nature of the tissue impedes optimal fixation.
- **Design:** Tumor FFPE tissues from breast cancer cases were retrieved between years 2000-2001. 34 cases were randomly selected for this pilot project based on morphological similarity and availability of tumor blocks with >75% tumor content. Total RNA was isolated (Recover-All Nucleic Acid Isolation Kit, Ambion), reverse transcribed (RT First Strand kit, Qiagen), and analyzed by real-time PCR on a Roche Lightcycler 480. Total RNA quantity was assessed on Nanodrop machine. Samples with more than 1ug of RNA yield were considered adequate for validation test with HPRT1 gene as primary screening housekeeping gene. Then the samples were run on Human Breast Cancer Signaling Array (Qiagen, PAH-131) that contained 84 key genes commonly involved in the dysregulation of signal transduction in breast cancer and 5 house keeping genes (Beta2 Microglobulin, GAPDH, HPRT1, ACTB, RPL13A). Positive calls were set at an arbitrary cycle threshold (CT) of 40 cycles.
- **Results:** Total RNA extractions yielded 65 ng to 18.75 ug RNA in 34 samples. 26/34 cases (77%) yielded more than 1ug of RNA and all showed successful HPRT1 gene amplification. 19/26 cases were run on the cancer signaling array. All cases had the housekeeping genes consistently amplified with CT of 26.6-38.7. Eighty-four breast cancer associated genes showed CT value ranging from 12.8-39.8 with most arrays yielding at least 65% positive calls.

- **Conclusions:** We have developed a protocol for the extraction and gene expression screening of decade old mRNA. While the cycle thresholds determined during the screen indicate either low relative expression or simply low recovery, the efficacy as a screening tool is readily apparent. Moreover, the process can go from paraffin block to usable screening mRNA expression data in a matter of a few hours. Applying this type of technology to additional breast data sets and other neoplasms will undoubtably increase the feasibility of using molecular techniques to retrieve valuable retrospective information currently locked away in every institution's FFPE storage archives.

  Category: Techniques